

DEPARTMENT OF HEALTH AND HUMAN SERVICES

MEMORANDUM OF CONFERENCE

March 17, 1995

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Subject: Glufosinate Tolerant Canola

Keywords: Canola; glufosinate (herbicide) tolerance; phosphinothricin acetyltransferase (PAT); neomycin phosphotransferase II (npt II; APH(3')II)

Introduction

The meeting was convened to bring to closure AgrEvo's consultation with FDA with regard to the food and feed safety of glufosinate tolerant canola HCN92. The line HCN92 contains the synthetic phosphinothricin acetyltransferase (*pat*) gene that confers glufosinate tolerance and the *npt II* gene that confers resistance to aminoglycoside antibiotics. AgrEvo previously had met with the agency on October 6, 1994 concerning this subject. In a letter dated February 27, 1995, AgrEvo submitted a summary of their safety assessment of glufosinate tolerant canola HCN92.

In a letter dated March 9, 1995, AgrEvo submitted a revised summary safety assessment (dated March 10, 1995) that had been edited to clarify the text. At the meeting that is the subject of this memorandum, representatives of AgrEvo described several errors that existed in the original and revised submissions. AgrEvo stated that the errors did not affect the conclusions from their safety assessment, and FDA personnel agreed that it was appropriate for AgrEvo to submit a revised copy of their safety assessment. AgrEvo's final revision was dated March 20, 1995.

Introduced Genetic Material

AgrEvo described the identity and function of the genetic material introduced into canola using the *Agrobacterium tumefaciens* mediated transformation system. AgrEvo presented data and information that allowed the firm to conclude that they have 1) demonstrated that there has not been transfer of plasmid DNA from outside the left or right border of the T-DNA region of the transformation vector, and that no additional coding sequences from the vector, other than the *pat* gene and the selectable marker gene (*npt II*), were incorporated into the plant genome as part of the transformation process; 2) determined that two copies of the introduced genetic material have been incorporated into a single locus in the plant genome (although there may have been some sequence alteration within one of the copies); and 3) determined that the insert remained stably integrated in the plant genome through successive generations.

Identity and Function of Expression Products encoded by the Inserted Genetic Material

Two new proteins (i.e., the enzymes phosphinothricin acetyltransferase (PAT) and neomycin phosphotransferase II (NPT II)) are expressed in canola HCN92.

AgrEvo stated that the PAT enzyme confers the glufosinate-tolerant phenotype on canola HCN92 because it acetylates glufosinate ammonium (L-phosphinothricin (PPT)), the active ingredient in broad spectrum, non-selective herbicides such as Liberty™, Harvest®, and Ignite®, rendering glufosinate ammonium inactive as an herbicide. AgrEvo also stated that acetyltransferases, which are found in nature in microbes, plants, and animals, have a common enzymatic function (transfer of an acetyl group from acetyl CoA to a substrate) but differ in substrate specificity. AgrEvo presented data concerning the temperature and Ph optima of PAT, the kinetics associated with PAT activity, and the substrate specificity of PAT.

AgrEvo also stated that HCN92 included the trait of tolerance to aminoglycoside antibiotics conferred by the selectable marker NPT II. AgrEvo presented data that allowed the firm to conclude that the NPT II protein expressed in canola HCN92 was the same as the bacterial NPT II protein. AgrEvo's submission did not further discuss the characteristics of the NPT II protein.

The agency has promulgated a regulation authorizing the use of the NPT II protein (APH(3')II) as a processing aid in the development of new varieties of, among other crops, oilseed rape (21 CFR 173.170 and 21 CFR 573.170).

Safety of the Introduced Protein

AgrEvo presented data that allowed the firm to conclude that PAT protein is not present in processed canola oil (which is the only food product used for human consumption) and that denatured (i.e., inactive) PAT protein is present in processed canola meal (which is used for animal feed) at a level of 2-5 ppm (0.005% of total protein in the meal). AgrEvo also presented data and information that allowed the firm to conclude that 1) the PAT protein is not known to be an allergen and does not bear sequence homology to known allergens or toxins; 2) PAT enzymatic activity is destroyed during the first stages of processing to produce edible oil and meal for animal feed use; and 3) PAT enzymatic activity is destroyed under simulated gastric conditions.

Compositional Analysis

Endogenous toxicants

In their submission, AgrEvo stated that 1) two components (i.e., erucic acid and glucosinolates) have been associated with nutritional and palatability concerns in rapeseed; 2) "canola" is a trademark term that is presently defined as seed, oil, and meal from *Brassica napus* and *B. rapa* plants that contain no more than 2% erucic acid in the seed oil and no more than 30 μ moles/g of aliphatic glucosinolates in the oil-free, moisture-free meal; and 3) a new variety of canola cannot be registered for use in Canada unless it contains less than 1% erucic acid in the seed oil and less than 20 μ moles/g glucosinolate(s) in the meal. AgrEvo presented data that allowed the firm to conclude that 1) erucic acid levels in canola HCN92 grown over multiple years and in multiple locations are lower than 1% and 2) glucosinolate levels in canola HCN92 grown over multiple years and in multiple locations are less than 20 μ moles/g. AgrEvo also presented data that allowed the firm to conclude that the levels of the anti-nutritive substance phytate were similar in HCN92 and commercially available canola lines.

Concentration of Important Nutrients

AgrEvo presented data that allowed the firm to conclude that the composition of canola HCN92 is equivalent to the composition of traditional canola. AgrEvo presented data concerning 1) seed composition (including oil, protein, ash, and crude fiber content) and 2) phytosterol levels (including tocopherols) in crude and refined oil.

Conclusions

AgrEvo has concluded, in essence, that the glufosinate tolerant canola HCN92 they have developed is not significantly altered within the meaning of 21 CFR 170.30(f)(2) when compared to canola varieties with a history of safe use. At this time, based on AgrEvo's description of its data and analysis, the agency considers AgrEvo's consultation on this product to be complete.

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